

REMARKS**Rejection under 35 U.S.C. 112, Second Paragraph**

The Office Action rejected claims 83-108 and 111-124 under 35 USC 112, second paragraph as indefinite for allegedly failing to particularly point out and distinctly claim the subject matter of the invention. In response, applicants respectfully traverse, and submit that the claims are sufficiently definite. The office action questions how a hairpin RNA that is already double stranded can be cleaved to further produce a double stranded product. While not being bound by the following, one can envision scenarios where there is cleavage at one end or both ends of a duplex region of a hairpin RNA species, yet still results in a double stranded product; or a hairpin RNA species wherein the region containing the hairpin turn is cleaved, leaving the complementary RNA double stranded product. Applicants submit that the claims are sufficiently definite and respectfully request reconsideration of this ground of rejection.

Rejections under 35 U.S.C. 103

The Office Action rejected claims 83-87, 90-98, 102-108, 111-115 and 120-124 under 35 USC 103(a) as allegedly unpatentable over Fire (US 6,506,559)(the "559 patent") and Ui-Tei (Febs Letters) as further evidenced by Zhang et al (Cell 2004). The Office Action also rejected claims 83-98, 101-108, and 111-124 under 35 USC 103(a) as allegedly unpatentable over Fire (the "559 patent"), Ui-Tei and Good (Gene Therapy 1997) as further evidenced by Zhang (Cell 2004). The Office Action further rejected claims 83-87, 90-98, 102-108, 111-115, and 120-124 as allegedly unpatentable over Fire and Ui-Tei. In response, applicants respectfully traverse.

The claimed method is not rendered obvious by the cited references either alone or in combination. Applicants submit that based on the literature as of the priority date, there was nothing to suggest attenuating expression of a target gene using the combination of the specific elements recited in the claims, including the use of a *library* of single stranded hairpin RNA species, and that

that the cell is both a mammalian cell, and suspended in culture. The combination recited in the claims would not have reasonably been made, if made at all, prior to applicant's invention, because prior to applicants' filing, those skilled in the art, including an inventor of the cited '559 patent, did not think one could attenuate expression with a library of RNA species using a RNAi mechanism in mammalian cells, let alone in mammalian cells suspended in culture, or understand the underlying mechanism for doing so. It was not until applicant's discovery of the underlying mechanism for RNA interference, as first described in applicants' specification, that those skilled in the art would have reasonably thought that RNAi could be applicable to mammalian cells, let alone mammalian cells suspended in culture. The basis for why one of skill in the art would have had no reasonable basis to arrive at applicants' claimed invention, prior to applicants' filing, is provided below.

i) mammalian cells

Applicants submit that prior to their invention, one of skill in the art would not have reasonably believed that a library of single stranded hairpin RNA species could induce sequence-specific gene silencing in mammalian cells. In order for those skilled in the art to have reasonably believed that a such RNA species could induce sequence-specific gene silencing, they first needed to understand the cellular mechanism of this biological phenomenon. At the time the '559 patent was filed in 1998, that mechanism was not known to the public nor described in the '559 patent, although procedures based on double stranded RNA-triggered silencing were fairly well-established tools for functional genomics of lower organisms (plants, invertebrates and fungi). Nevertheless, the simple protocols used for invertebrate and plant systems were not effective in mammalian cells at that time. Indeed, an article by Andrew Fire (a named inventor of the '559 patent) published after the filing date of the '559 patent, provides evidence that based on the '559 patent, one skilled in the art would had no reasonable basis to arrive at applicants' claims, which recite, *inter alia*, "mammalian cell." (Fire, *Trends in Genetics*, 15: 358-363 (1999) (the "Fire Article"))¹:

Procedures based on RNA-triggered silencing are now well-established tools for functional genomics of lower organisms (plants, invertebrates and fungi). Valuable information about gene function can be obtained, even in cases where only a partial loss-of-function is generated. From a technical perspective, one could certainly hope

¹ The reference was already made of record (reference AU) in an information disclosure statement filed May 30, 2003. Nevertheless, applicants are enclosing another copy of this reference for the Examiner's convenience.

that RNA-triggered silencing would exist in vertebrates: this would facilitate functional genomics and might allow medical applications involving targeted gene silencing of 'renegade' genes. Although this hope is not ruled out by any current data, the simple protocols used for invertebrate and plant systems are unlikely to be effective. Mammals have a vehement response to dsRNA, the best-characterized component of which is a protein kinase (PKR) that responds to dsRNA by phosphorylating (and inactivating) translation factor EIF2a.*** (Fire Article, pages 362-363)

The Fire Article further states that "[a]ny gene-specific dsRNA response in mammals would need to exist in cells or conditions where PKR is less effective, or would need to work in the shadow of the PKR-induced global response." See Fire Article, page 363. Accordingly, even as late as September 1999 (*i.e.* later than the filing date of the '559 patent), Andrew Fire, one of the named inventors of the '559 patent, essentially conceded that understanding the underlying mechanism of using dsRNA to induce sequence specific gene silencing in mammalian cells was important, and his statements support the notion that he in fact did not understand such mechanism at the time.

Additionally, Wianny et al. (Nature Cell Biology) ("Wianny")², a reference published after the filing date of the '559 patent, provides further evidence that those skilled in the art did not believe gene attenuation using RNAi in mammalian cells could be accomplished prior to applicants' invention. In particular, Wianny states that "[s]o far there has been no report that RNAi can be used in mammals. Moreover, there are several indications of potential limitations to its function in this group of animals. Principal among these is that the accumulation of very small amounts of dsRNA in mammalian cells following viral infection results in the interferon response, which leads to an overall block to translation and the onset of apoptosis. Such considerations have discouraged investigators from using RNAi in mammals" (page 71, left column, lines 4-12).

Wianny further states that

Thus it appears that the concerns that RNAi might not work in the mouse may have been raised prematurely. Concern has been expressed that the protocols used for invertebrate and plant systems are unlikely to be effective in mammals, because accumulation of dsRNA in

² The reference was already made of record (reference CJ) in an information disclosure statement filed May 30, 2003. Nevertheless, applicants are enclosing another copy of this reference for the Examiner's convenience.

mammalian cells can result in a general blockage of protein synthesis. The presence of extremely low concentrations of dsRNA in viral infections triggers the interferon response, part of which is the activation of a dsRNA responsive protein kinase (PKR). This enzyme phosphorylates and inactivates translation factor EIF2 α in response to dsRNA. The consequence is a global suppression of translation, which in turn triggers apoptosis. However, we have shown here that the injection of a dsRNA is specific to the corresponding gene; it does not cause a general translational arrest, because embryos continue to develop and we see no signs of cell death. It is possible that the early mouse embryo is incapable of an interferon response, *and that there still may be difficulties in using RNAi at later stages.* [emphasis added]

Accordingly, Wianny provides further evidence that the literature suggested that one skilled in the art would have had no reasonable basis to arrive at applicants' claimed invention as of February 2000 (*i.e.*, Wianny's publication date), let alone as of the filing date of the '559 patent, both of which are earlier than applicants' filing date.

Thus, in the absence of the biochemical and genetic approaches carried out by the inventors in several experimental systems and described in applicants' specification, those skilled in the art would have had no reasonable expectation that, based on the teachings of the '559 patent, single self-complementary RNA strands would have any effect as a gene silencing agent in mammalian cells. Moreover, prior to applicants' invention, one skilled in the art would not have believed that the claimed invention would work. *Applicants respectfully submit that the only way that one could read the '559 patent as teaching a dsRNA to attenuate expression in a mammalian cell in culture would be if one did not understand the literature; that is, if one were not one skilled the art.* Applicants further submit that the understanding of the mechanisms underlying RNAi in vertebrates came from the work of the present inventors, who identified the existence of conserved machinery for double stranded RNA-induced gene silencing from drosophila to mammals. The present inventors also defined the RNAi process as proceeding via a two-step mechanism. In the first step, double stranded RNA is recognized by an RNase III family nuclease called Dicer, which cleaves the dsRNA into about 21-23-nt siRNAs (now called "small interfering RNA" or "siRNA" in the scientific literature). These siRNAs are incorporated into a multicomponent nuclease complex (RISC), which identifies substrates through their homology to siRNAs and targets these cognate mRNAs for destruction. Accordingly, based on the comments provided above, applicants submit

that one skilled in the art would have had no reasonable basis to think that attenuation of gene expression RNAi would work in mammalian cells, based on the disclosure of the '559 patent, before applicants' filing.

ii) suspension in culture

Applicants submit that at the time the '599 patent was filed, one skilled in the art would not have reasonably believed that one could inhibit expression of an endogenous genomic gene with an RNAi mechanism in cells suspended in culture. In particular, at the time the '559 patent was filed, the ability of a few molecules of double stranded RNA to eliminate a much larger pool of endogenous mRNA had suggested a catalytic or amplification component to the interference mechanism. For instance, some of the plant literature favored an RNA-based copying system that was proposed to produce copious amounts of antisense RNA (while perhaps also producing additional sense and dsRNA). See Jorgensen et al. (1998) *Science* 279: 1486; Waterhouse et al. (1998) *Proc. Natl. Acad. Sci.* 95:13959 and Wassenegger et al. (1998) *Plant Mol. Biol.* 37:349. This experience, along with similar observations in *C. elegans* (see, e.g., Fire et al. (1998) *Nature* 391:806), suggested an amplification process in whole organisms. That is, that RNA interference was the consequence of a systemic response. *If that had indeed been the mechanism, it would not be apparent or expected that RNA interference using double stranded RNA would work on cultured cells.* Accordingly, Applicants submit that one skilled in the art would not have expected to achieve attenuation of gene expression in cells suspended in culture using an RNAi mechanism.

In sum, Applicants submit that based on the literature as of applicants' filing date, there was nothing to suggest that one skilled in the art would have been able to attenuate expression, with any reasonable expectation of success, of one or more target genes using the combination of the specific elements recited in the claims, including that the use of a library of single stranded hairpin RNA species, and that the cell is (i) a mammalian cell, and (ii) is suspended in culture. Accordingly, applicants' claimed invention is not obvious over the cited references. Applicants respectfully request reconsideration.

Rejection under 35 USC 103(a)

The Office Action rejected claims 83-88, 90-108 and 113-124 under 35 U.S.C. 103(a) as being unpatentable over Lieber (US 6,130,092) and Tuschl (2002/0086356), Kennerdel (Nature 2000), Fire (US 6,506,559) and Barber (US 6,605,429). In response, applicants respectfully traverse. First, applicants note that they have amended the claims to more clearly point out and distinctly claim the subject matter of their invention. In addition, applicants note that the office action states that Barber and Lieber both relate to *ribozymes*. It is well known that ribozymes are enzymatic nucleic acids containing two types of sequences: those that bind to the target sequence, and those with secondary structure forming a catalytic core. Ribozyme sequences that bind to the target nucleic acid are not complementary to another sequence within the ribozyme. Ribozymes do not contain sequences that both bind to the target sequence, and are complementary to another sequence within the ribozyme molecule. In contrast, the present claims recite a hairpin RNA comprising self complementary sequences that form duplex regions *and* which hybridize to the target gene. The hairpin RNAs of the instant application therefore comprise sequences that are complementary to both the target gene and another sequence within the hairpin RNA molecule itself. As such, the hairpin RNAs of the instant application are distinct from the ribozyme molecules described in Barber and Lieber. Applicants submit that there was no motivation to combine either Barber or Lieber (relating to ribozymes) with the other cited references relating to RNAi (such as the siRNAs disclosed in Tuschl), let alone to arrive at applicants' claimed invention with any reasonable expectation of success. The other cited references do not supply what is missing from Barber and/or Lieber. Accordingly, the cited references either alone or in combination do not render obvious the claimed invention. Applicants respectfully request reconsideration.

Rejection under 35 USC 102

The Office Action rejected claims 83-88, 90-100, 102-108, 113-115m 120, 123-124 under 35 USC 102(e) as being anticipated by Barber (US 6,605,429) as evidenced by Hammond (Nature 2000). In response, applicants respectfully traverse. Applicants note that Barber relates to ribozyme constructs. The Office Action states that the claims did not explicitly state that the duplex regions

hybridize to the target gene. In accordance with the Examiner's suggestion, applicants have herein amended the claims such that they more clearly recite that the duplex region hybridizes under intracellular conditions to a target gene. Applicants submit that Barber does not anticipate the claimed invention because ribozymes do not contain sequences that both bind to the target sequence, and are complementary to another sequence within the ribozyme molecule. Applicants respectfully request reconsideration.

Rejection under 35 USC 103(a)

The Office Action rejected claims 83-88, 90-100, 102-108, 113-115m 120, 123-124 under 35 USC 103 as being unpatentable over Barber (US 6,605,429) in view of Good (Gene Therapy), Lipardi (Cell 2001) in further view of Bennett (US 5,998,148) and as evidenced by Hammond (Nature). In response, applicants respectfully traverse. As stated above, Applicants note that Barber relates to ribozyme constructs. The Office Action states that the claims did not explicitly state that the duplex regions hybridize to the target gene. In accordance with the Examiner's suggestion, applicants have herein amended the claims such that they more clearly recite that the duplex region hybridizes under intracellular conditions to a target gene. Accordingly, Barber either alone or in combination does not render obvious the claimed invention. The other references do not supply what is missing from Barber. There was no motivation to combine Barber with the other cited references relating to RNAi, let alone to arrive at applicants' claimed invention with any reasonable expectation of success. The other cited references do not supply what is missing from Barber. Accordingly, the cited references either alone or in combination do not render obvious the claimed invention.

In view of the above amendment, applicant believes the pending application is in condition for allowance.